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**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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In re Application of: SIEGALL ET AL.

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| Serial No.: 09/328,296 | Examiner: Karen Canella |
| Filed: June 8, 1999 | Group Art Unit: 1642 |
| For: RECOMBINANT ANTI-CD40 ANTIBODY AND USES THEREOF | Attorney Docket No.: 9632-005 |

**DECLARATION OF DR. JOSEPH A. FRANCISCO UNDER 37 C.F.R. § 1.132**

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Joseph A. Francisco, do declare and state that:

1. I am a citizen of the United States residing at 21705 92nd Avenue W., Edmonds, Washington 98020.

2. I am an inventor of the invention described and claimed in the above-identified patent application, Serial No. 09/328,296.

3. From 1998 to the present, I have been at Seattle Genetics, Inc., assignee of the above-identified application, initially as a Principal Scientist (from 1998 to 2000) and presently as Associate Director of Molecular Biology. From 1997 to 1998, I occupied a Scientist position at Monsanto in Saint Louis, Missouri. From 1994 to 1997 I was at Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, Washington, first as a Postdoctoral Fellow (1994-

1996), then as a Research Investigator (1997). I received the degree of Doctor of Philosophy from the University of Texas at Austin in Chemical Engineering in 1994.

4. My academic and technical experience and honors, and a list of my publications are set forth in my *curriculum vitae,* attached hereto as Exhibit 1.

5. The experiments described in paragraphs 6 through 8.4 hereinbelow were carried out by me or under my supervision.

6. A recombinant hybridoma expressing chimeric S2C6 (cS2C6), *i.e.*, a derivative of S2C6 containing the variable regions of S2C6 and human constant regions, was constructed in accordance with the principles of chimeric antibody construction taught on page 25, line 32 through page 26, line 15 of the specification of U.S. application 09/328,296 and using routine methods known in the art.

6.1. The vectors and method utilized in the construction of the cS2C6 expression constructs were originally described in Yarnold and Fell, 1994, Cancer Research 54:506-512 and U.S. Patent No. 5,482,856.

6.2. The particular vectors utilized for construction of an cS2C6 expression construct were designed to replace the murine antibody heavy and light chain constant regions with the human gamma 1 and kappa constant regions, respectively, via homologous recombination, while maintaining the original S2C6 heavy and light chain variable regions.

6.3. The first step involved replacing the gene segment coding for the murine gamma 1 constant region with the analogous gene segment coding for the human gamma 1 constant region using the heavy chain conversion vector shown in Exhibit 2. As stated in U.S. Patent No. 5,482,856, this vector

was deposited with Northern Regional Research Center ("NRRL") and assigned accession number B-18599.

6.3.1. The heavy chain conversion vector contains the human gamma 1 constant region gene - CH1, hinge, CH2 and CH3 - and the neomycin resistance gene flanked by regions that are homologous to the murine Ig locus. The vector also contains the ampicillin resistance gene and the pUC19 origin of replication for maintenance and plasmid replication in *E. coli.*

6.3.2. The heavy chain conversion vector was linearized by restriction digestion and transfected by electroporation into the S2C6 hybridoma.

6.3.3. The transfected cells were then plated into 96-well microtiter plates in growth media containing neomycin. Only those cells which incorporated the neomycin resistance gene, and therefore the human gamma 1 heavy chain gene as well, are capable of growing in the presence of neomycin.

6.3.4. After approximately 3 weeks of growth the cells were screened by ELISA for the presence of the human IgG1 heavy chain. The resulting clones expressed a chimeric heavy chain consisting of the murine heavy chain variable region joined to the human gamma 1 heavy chain constant regions.

6.3.5. At this stage the clones still expressed an entirely murine light chain. The clones that expressed the highest level of human

heavy chain, as determined by ELISA, were selected for transfection with the light chain vector.

6.4. Chimerization of the light chain was accomplished in a similar manner using the light chain chimerization vector shown in Exhibit 3. As stated in U.S. Patent No. 5,482,856, this vector was deposited with NRRL and assigned accession number B-18597.

6.4.1. The light chain conversion vector contains the human kappa constant region and the xgpt (xanthine-guanine phosphoribosyltransferase) gene, which provides resistance to mycophenolic acid, flanked by regions that are homologous to areas upstream and downstream of the constant region in the mouse kappa locus.

6.4.2. As with the heavy chain vector, the light chain vector also contains the ampicillin resistance gene and the pUC19 origin of replication for maintenance and plasmid replication in *E. coli*.

6.4.3. The light chain vector was linearized by restriction digestion and the vector was transfected into the clones described above.

6.4.4. The transfected cells were then plated into 96-well microtiter plates in media that contained mycophenolic acid. Only those cells which incorporated the xgpt gene, and therefore the human kappa constant region, are capable of growing in the presence of mycophenolic acid.

6.4.5. The cells were then tested by ELISA for the presence of both human heavy chain and light chain constant regions. The clone

that expressed the highest level of chimeric antibody, that is, antibody containing the murine heavy and light chain variable regions and the human gamma 1 and kappa constant regions, was identified.

6.5. The clone identified in ¶ 6.4.5, *supra*, was then expanded and used for producing chimeric antibody for subsequent studies.

7. The binding activity of cS2C6 was compared to that of the parental murine antibody in a competition fluorescence activated cell sorting (FACS) experiment using Raji non-Hodgkin's lymphoma cells, which are known to express CD40.

7.1. Raji non-Hodgkin's lymphoma cells were incubated with 0.2 μg/ml murine S2C6 conjugated to the fluorescent dye phycoerythrin (S2C6-PE), and 10 μg/ml of murine S2C6, cS2C6, or a non-binding control antibody.

7.2. The cells were then washed and evaluated on a FACScan fluorescence activated cell sorter.

7.3. As shown in the Exhibit 4A, cells incubated with S2C6-PE (represented by the empty histogram outlined by the solid line) showed an increase in fluorescence intensity compared to unlabeled cells (represented by the histogram outlined with the dashed line), indicating that the fluorescent-labeled antibody specifically bound to the cells.

7.4. When an excess of non-binding control antibody was incubated with the cells in the presence of the labeled S2C6, shown in Exhibit 4B, the fluorescence signal was identical to that shown in Exhibit 4A for S2C6-

PE-bound Raji cells in the absence of competitor, indicating that the irrelevant control antibody did not compete for binding to CD40.

7.5. In contrast, equivalent levels of either unlabeled murine S2C6 (Exhibit 4C, shaded histogram) or unlabeled cS2C6 (Exhibit 4D, shaded histogram) completely inhibited the S2C6-PE from binding to the cells.

7.6. The fluorescent signal seen in both of these cases was equivalent to that seen with the unlabeled cells in Exhibit 4A. The S2C6-PE histogram from Exhibit 4A is shown for comparison with Exhibit 4, panels C and D.

7.7. These results indicate that the binding activity of the chimeric antibody to Raji cells is equivalent to the parental murine antibody.

8. The *in vivo* efficacy of cS2C6 was evaluated in a disseminated non-Hodgkin's lymphoma (NHL) xenograft model in SCID mice. SCID mice were injected through the tail vein with $1x10^6$ Ramos NHL cells.

8.1. The mice were then divided into groups that received either no treatment, cS2C6 twice per week at 2.0 mg/kg for a total of 5 injection, or a isotype matched control chimeric antibody twice per week at 2.0 mg/kg for a total of 5 injections.

8.2. In this model untreated animals or those that receive an ineffective therapy develop a severe disseminated disease which manifests through a variety of signs, including complete hind limb paralysis.

8.3. As shown in Exhibit 5 below, untreated animals and animals receiving control antibody developed severe disseminated disease within 34 days of tumor cell injection, at which point they were sacrificed.

8.4. In contrast, 7 of 8 animals that received cS2C6 survived for the entire length of the study, 141 days, with no signs of disease, indicating that the chimeric antibody was efficacious in preventing NHL.

9. The results of the experiments described above demonstrate that chimeric S2C6 has a binding affinity to CD40 that is similar to that of the parental monoclonal antibody S2C6, and is functionally equivalent to S2C6.

10. I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: April 27, 2001

Joseph A. Francisco

Attachments:

Exhibit 1: *Curriculum Vitae* of Joseph A. Francisco

Exhibit 2: The heavy chain conversion vector

Exhibit 3: The light chain conversion vector

Exhibit 4: Figure entitled "Competition FACS with cS2C6"

Exhibit 5: Figure entitled "*In vivo* efficacy of cS2C6"